Antimicrobial activities and total phenolic contents of the sap of Pycnanthus angolensis and root of Cryptolepis sanguinolenta

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Abstract

The death of many people in tropical countries can be attributed to microbial infection, probably, because synthetic antibiotics are failing in the treatment of these microbial infections, attributed to the ability of the microorganisms to mutate and adapt to harsh conditions. This study evaluated, in vitro, the antimicrobial activities, antioxidant potentials, and the total phenolic contents of aqueous and ethanol extracts of the root of Cryptolepis sanguinolenta (Lindl.) and the crude sap of Pycnanthus angolensis (Welw) using selected standard bacteria strains (S. aureus (ATCC 25923), S. saprophyticus (ATCC 15305), E. coli (ATCC 25922), S. typhi (ATCC 19430), P. aeruginosa (ATCC 27853), and P. mirabilis (ATCC 49565). The modified agar well diffusion method was used to evaluate the antimicrobial activities of the plant extracts. Chloramphenicol and tetracycline were used as positive controls. The extracts were also screened for specific phytochemicals and using the Folin Ciocalteu reagent test, their total phenolic contents were determined. The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, and saponins in both Cryptolepis sanguinolenta and Pycnanthus angolensis. For the antimicrobial activities, all the test bacteria were susceptible to the crude sap of Pycnanthus angolensis except P. mirabilis. In the case of the Cryptolepis sanguinolenta, only S. aureus was susceptible to both aqueous and ethanol extracts. The total phenolic content, expressed in g/100g GAE, recorded values of 55.427 ± 4.248 for the crude sap of Pycnanthus angolensis, and 11.642 ± 4.248 and 26.888 ± 4.248 for the aqueous and ethanol extracts of Cryptolepis sanguinolenta, respectively. It was concluded that Cryptolepis sanguinolenta and Pycnanthus angolensis are excellent candidates for further development of antimicrobial agents in the fight against microbial infections given the pressing need for novel efficacious agents.

Introduction

Humans and animals have coexisted with plants since the beginning of time, using them for food and medicine for thousands of years (1). According to the World Health Organization (2), the use of traditional medicine in treating diseases is based on health practices, knowledge, and beliefs in incorporating plants, animals, and mineral-based, spiritual therapies that are applied solely in the prevention and treatment of diseases and illness (2).

Despite the significant advances observed in modern medicine, herbal medications have been used to relieve the symptoms of various diseases (3). For many years, most synthetic antibiotics have been inefficient in treating microbial illnesses and this is due to the ability of microbial genes to mutate and resist the effect of antibiotics (3). The interest in medicinal plants in treating diseases is due to their prolonged use by our forefathers in treating themselves, a term known as "traditional" or "indigenous" medicines (2, 4).

Pycnanthus angolensis (P. angolensis) is a nutmeg tree species in the Myristicaceae family. In Africa, it is widely known as “Ilomba” (5–7). The Twi and Fante dialects of Ghana call it by the name “Otie”. It serves numerous purposes. For instance, the dried fruits of P. angolensis are used as spices for soups (4). The
seed yields a reddish-yellow brown fat known as Kombo butter or Angola tallow, which is used for illumination and soap making (8). The bark is used for treating skin infections, as a purgative, cleansing the milk of lactating mothers, and treating chest and cough pains. In Ghana, the bark is used to treat anaemia, while in Côte D’Ivoire, it is used as an antidote against ascites and leprosy (5–7, 9). In Congo DR, the bark is used to solve infertility problems and treat gonorrhoea and malaria. Antimicrobial and anthelmintic properties of the leaves, stem and roots have also been reported (7). The leaves also possess antioxidant and anti-inflammatory activities (10) and this has necessitated the need to discover the antimicrobial and antioxidant activities of the crude sap extract of \textit{P. angolensis} (6).

\textit{Cryptolepis sanguinolenta (C. sanguinolenta)} is the most familiar plant of the species of trees belonging to the family Apocynaceae (11, 12). It is commonly called “Nibima” in Ghana (12) and serves numerous medicinal purposes, including herbalists' use in treating fever, urinary tract infections, and upper respiratory tract infections (13, 14). The most well-known use of \textit{C. sanguinolenta} roots is in the treatment of malaria (15, 16). It is also historically used to treat insomnia, although the mechanism underlying this has yet to be fully understood (17). Despite the prevalent use of \textit{P. angolensis} and \textit{C. sanguinolenta} since time immemorial, much knowledge has yet to be found in the literature evaluating their antimicrobial and antioxidative properties (11, 18–21). The current study, therefore, seeks to evaluate in vitro the antimicrobial, phytoconstituents, total phenols, and antioxidant activity of the crude sap of \textit{P. angolensis} and the roots of \textit{C. sanguinolenta}.

**Materials And Method**

**2.1. Crude plants collection**

\textit{P. angolensis} was obtained at Forig, on the Okodee road, Bungalow number 15, Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi (Ashanti Region, Ghana), and the root of \textit{C. sanguinolenta} (specimen voucher number: CSRPM 1911) was obtained at the Centre for Plant Medicine Research Institute (CPMR) in Akuapim-Mampong (Ghana).

Plants’ samples were authenticated by Mr Papa Kofi a botanist at KNUST, and deposited to the herbarium of the CPMR, at Akuapim-Mampong. The sap of \textit{P. angolensis} was obtained by cutting or making a 45-degree angle in the stem of the tree plant, and the sap was collected using a sterilized 50 mL centrifuge tube. The crude branch was freeze-dried and stored at 4 °C for further downstream analysis.

The fresh root of \textit{C. sanguinolenta} was washed 2–3 times for 10 minutes with running tap water and once with sterile water, then shade-dried at room temperature until they were dried. The root was homogenized to a fine powder and stored in airtight bags for further downstream analysis (12).

**2.2. Areas for Analysis**

The in vitro evaluations (antioxidants and total phenolic contents) were carried out at the Noguchi Memorial Institute for Medical Research (NMIMR) in Accra, Ghana, and all phytochemical screenings and
in vitro analysis of antimicrobial activities were carried out at the Microbiology laboratory in the Department of Biochemistry and Biotechnology, KNUST.

2.3. Ethanol extract preparation of the root of Cryptolepis sanguinolenta

A mass of 700 g of dried powdered C. sanguinolenta roots was taken and dissolved in 7 L of 70% ethanol at room temperature for 48 hours. The resulting extract was filtered through a two-fold muslin cloth followed by Whatman No. 1 paper, the filtrate was condensed at 65 0C using a rotary evaporator (Buchi Rotavapor R-100, Switzerland), and freeze-dried. The freeze-dried material was stored at 4 0C in the refrigerator for further downstream analysis (22).

2.4. Aqueous extract preparation of the root of Cryptolepis sanguinolenta

The powdered root (800 g) was first soaked in distilled water (8 L) for 30 minutes, followed by 30 minutes of boiling (100 0C) to produce the aqueous extract. The boiled mixture was allowed to simmer for 30 minutes to evaporate the excess water. The remaining extract was then freeze-dried for further downstream analysis (22).

2.5. Utilised test microorganisms

The test organisms utilised in this study came from the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. The CSRPM used biochemical and Analytical Profile Index (API) assays to validate the existence of these species. Staphylococcus aureus (ATCC 25923), Staphylococcus saprophyticus (ATCC 15305), Proteus mirabilis (ATCC 49565), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), and Salmonella typhi (ATCC 19430) were the standard microbes used.

2.6. Inoculum preparation

The National Committee for Clinical Laboratory Standards (2003) technique for inoculum production was slightly modified to prepare the bacterial culture for this study (23). Stock cultures of the test bacterial strains, S. aureus (ATCC 25923), S. saprophyticus (ATCC 15305) E. coli (ATCC 25922), S. typhi (ATCC 19430), P. aeruginosa (ATCC 27853), and P. mirabilis (ATCC 49565) were streaked onto fresh nutrient agar plates and incubated to obtain isolated colonies. Four to five well-isolated colonies were transferred with an inoculating loop into 5 mL Mueller-Hinton broth and incubated at 37 0C for 16–24 hours until turbid. The turbidity was adjusted by adding sterile broth to attain the 0.5 McFarland turbidity standard (McFarland 0.5 equals approximately 10^8 CFU/mL) (23).

2.7. Antimicrobial assay

The National Committee for Clinical Laboratory Standards (2003) (23) method of the agar well diffusion method was used to determine the antimicrobial property of the sap and root extracts (16). A sterile cotton swab was dipped into the suspension within 15 minutes after adjusting the turbidity of the
inoculum suspension. Pressing firmly against the inside wall of the tube just above the fluid level, the swab was rotated to remove excess liquid. The swab was streaked over the entire surface of the Mueller-Hinton agar three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums and allowed to dry. A sterile cork borer was used to make holes in the dried medium. Approximately 100 µL of 32 mg/mL of each extract was micro-pipetted into the wells/holes labelled. Standard drugs, 30 µg/mL chloramphenicol and tetracycline were placed in respective wells/holes as a positive control for bacteria and 100 µL of 20% v/v of DMSO was used as a negative control. Three other replica plates were for each plate of a particular test microbe and incubated at 35 °C for 24 hours. After incubation, the diameters of the zones of inhibition were measured with a sterilized ruler (23).

2.8. Solubility tests

An appreciable amount of the various extracts each were treated with water, absolute ethanol, and 50% ethanol, respectively, and vortexed for a few minutes to qualitatively determine which solvent the various samples are best soluble in.

2.9. Determination of total phenol content

The total phenolic content of the extracts was assessed using a modified Gustafson et al. (2012) (24) technique and the Folin-Ciocalteu reagent. A concentration of 5 mg/mL of pyro-gallic acid solution (which was prepared by diluting 5 mg of pyro-gallic acid powder with 100 µL of absolute ethanol and 900 µL of distilled water), which was diluted to 10 different concentrations using a two-fold dilution served as the reference standard for the analysis. A mass of 5 mg of the extracts was treated with 1 mL of solvent obtained from their respective solubilities. The individual solutions were then diluted to three different concentrations using a two-fold dilution. For the assay, 10 µL of pyro-gallic acid solution and extract solutions prepared in triplicate were treated with 1 µL of distilled water in a 24-well plate. A 50 µL of Folin-Ciocalteu reagent (Folin: Methanol, 1:1, v/v) was added to each dilution. The samples were wrapped in foil and incubated in the dark for five minutes, after which 150 µL of Na₂CO₃ was added to the respective dilutions and incubated again at room temperature for two hours. Absorbance was then measured using a microplate spectrophotometer (Tecan Infinite M200 Pro Plate Reader, Austria) at 750 nm against a blank comprised of the various solvents and reagents minus the extracts (standard). The mean value absorbance of the standard was then obtained, and a calibration line was drawn. The concentration of phenolic content in the various extracts was then extrapolated from the graph (24).

2.10. Determination of antioxidant activity

The free radical scavenging activity of the extracts based on the scavenging activity of the stable DPPH free radical was determined by the method described by Sochor et al. (25) with modifications. A concentration of 1 mg/mL of BHT solution (prepared by diluting 1 mg of BHT powder with 1 mL methanol) served as the standard for the analysis. A 0.5 mM DPPH solution was prepared by dissolving 5 mg of DPPH powder in 25 mL of methanol. A mass of 40 mg of the individual extracts was treated with 1
mL of the solvent obtained from their respective solubility tests. The stock solutions were then diluted to 7 different concentrations using a three-fold dilution. The standard stock was also diluted to 7 different concentrations using a two-fold dilution. A colour control (made up of the sample and methanol only but not DPPH) was also carried out for both the standard and the extracts. The colour control for the extracts was prepared in a three-fold dilution to obtain seven different concentrations.

In contrast, the colour control for BHT was prepared in a two-fold dilution to obtain seven different concentrations. For the assay, a volume of 100 µL of each of the extracts and standard concentrations was treated with 100 µL of DPPH solution in a 96-well plate, shaken vigorously, wrapped in foil, and incubated in the dark at room temperature for 20 minutes, after which the absorbances of all the contents of the plate were read using a microplate spectrophotometer (Tecan Infinite M200 Pro Plate Reader, Austria) at 517 nm of wavelength against their respective blank solutions. All experiments were performed in triplicates and the percentage scavenging activity of the DPPH-free radical was calculated using the formula:

\[
\% \text{ Scavenging activity} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)} \times 100}{\text{Absorbance (Control)}}
\]

Where Absorbance (control) = blank – colour control

Absorbance (sample) = mean absorbance of triplicate – colour control

A graph of mean percentage antioxidant activity against the concentrations of the various extracts was then plotted and the IC\(_{50}\) was calculated using a gradient (25).

2.11 Phytochemical screening

The phytochemical analysis was performed on both aqueous and ethanolic extracts of the roots and crude sap using the standard procedures of Edeoga et al. (26). The foam test for saponins, Wagner’s test for alkaloids, Braymer’s test for tannins, the alkaline reagent and sodium hydroxide test for flavonoids, the ferric chloride test for phenols, Keller Kelliani’s test for cardiac glycosides and Salkowski’s test for terpenoids were carried out (26).

2.11.1 Test for Alkaloids (Wagner’s test)

The crude sap and root extracts were treated separately with 3.5 drops of Wagner’s reagents (1.27 g of iodine and 2 g of potassium iodide in 100 mL of water). The formation of a reddish-brown inference indicated the presence of alkaloids in crude extracts.

2.11.2 Test for Saponins (foam test)

A gram of the sap and the root extracts were placed in different test tubes and 2.5 mL of distilled water was added. The mixtures were boiled and filtered. The filtrate was mixed with 3 mL of distilled water and vigorously shaken for about 5 minutes. The formation of a persistent froth indicated the presence of saponins in the plant sample.
2.11.3 Test for Terpenoids (Salkowki’s tests)
A volume of 1 mL of chloroform was added to 1 mL of the crude sap and root extracts, followed by a few drops of concentrated Sulphuric acid ($H_2SO_4$). The sample was observed for a reddish-brown colouration to draw an interface that indicates the presence of terpenoids.

2.11.4 Test for Tannins (Braymer’s test).
A volume of 1 mL of the sap and root extracts was treated with a 10% alcoholic ferric chloride solution. The formation of brownish green or a blue-black colouration showed the presence of tannins.

2.11.5 Test for Flavonoids (alkaline reagents and sodium hydroxide test).
A volume of 1 mL of the sap and root extracts was treated with drops of a 20% sodium hydroxide (NaOH) solution. The formation of a persistent froth indicated the presence of saponins in the plant sample.

2.11.6 Test for Phenols (the ferric chloride test).
A 5% aqueous ferric chloride was used to treat a portion of the crude sap and root extracts and the formation of a deep blue or black colouration indicated the presence of phenols.

2.11.7 Test for Cardiac Glycosides (Keller Kelliani’s test).
In test tubes, 1 mL of glacial acetic acid was added to 2.5 mL of sap and root extracts, followed by a drop of ferric chlorides. A volume of 1 mL of concentrated Sulphuric acid ($H_2SO_4$) was added and the formation of a brown ring at the interface indicated the presence of deoxy-sugar characteristic of cardenolides (26).

2.12 Statistical Analysis
All grouped data were statistically analysed using Microsoft Excel 2010 and Graph Pad Prism version 8. One-way ANOVA was used for the hypothesis testing where a p-value of less than 0.05 was considered to indicate statistical significance.

Results

In vitro antibacterial assay

Tables 1–5 show the in vitro antimicrobial screening derived from crude extracts of *P. angolensis* sap and *C. sanguinolenta* root. The *P. angolensis* inhibited the growth of all the test bacteria except *P. mirabilis*, while *C. sanguinolenta* could not inhibit any test bacteria except *S. aureus*. Table 4 represents chloramphenicol and tetracycline (standard antibiotics), and Table 5 describes the antibacterial activities of 20% DMSO (a negative control). Chloramphenicol and tetracycline showed activity against all the bacteria tested. Overall, the standard antibiotics showed the highest antibiotic activities as indicated in Table 4.
Table 1
Susceptibility of the test microbes to the crude sap of *P. angolensis*

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>ZONES OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>S. typhi</td>
<td>10.00 ± 0.00</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>10.00 ± 0.00</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10.50 ± 0.50</td>
</tr>
<tr>
<td>P. aeuroginosa</td>
<td>10.50 ± 0.50</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli</td>
<td>10.50 ± 0.50</td>
</tr>
</tbody>
</table>

The antimicrobial activity screening of aqueous and ethanolic extracts of *C. sanguinolenta* gave a 12.5% susceptibility with only *S. aureus* being the only microbe which showed some level of susceptibility.

Table 2
Susceptibility of the test microbes to the ethanol extracts of *C. sanguinolenta*

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>ZONES OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>S. typhi</td>
<td>NS</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>NS</td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.50 ± 0.50</td>
</tr>
<tr>
<td>P. aeuroginosa</td>
<td>NS</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table 3
Susceptibility of the test microbes to the aqueous extracts of *C. sanguinolenta*

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>ZONES OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>S. typhi</td>
<td>NS</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>NS</td>
</tr>
<tr>
<td>S. aureus</td>
<td>12.00 ± 0.00</td>
</tr>
<tr>
<td>P. aeuroginosa</td>
<td>NS</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli</td>
<td>NS</td>
</tr>
</tbody>
</table>

The *in vitro* antibacterial screening results obtained from tetracycline and chloramphenicol showed that all the test microbes were susceptible to the antibiotics, with *P. mirabilis* showing the least inhibition to tetracycline (39.00 ± 0.00) and *S. aureus* (48.00 ± 0.00) showing the highest inhibition for tetracycline (Table 4). For the chloramphenicol, *S. typhi* (15.00 ± 0.00) showed the lowest inhibition, while *P. aeuroginosa* (33.00 ± 0.00) showed the highest inhibition.

The potency of the concentrations of the extracts against *S. aureus* showed a trend with a positive gradient (Fig. 1). The activity increased with increasing concentration thus, the highest concentration (30%) exhibited the highest antimicrobial activity.

Table 4
Susceptibility of test microbes to the tetracycline and chloramphenicol

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>ZONES OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tetracycline</td>
</tr>
<tr>
<td>S. typhi</td>
<td>44.00 ± 0.00</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>47.00 ± 0.00</td>
</tr>
<tr>
<td>S. aureus</td>
<td>48.00 ± 0.00</td>
</tr>
<tr>
<td>P. aeuroginosa</td>
<td>45.00 ± 0.00</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>39.00 ± 0.00</td>
</tr>
<tr>
<td>E. coli</td>
<td>41.00 ± 0.00</td>
</tr>
</tbody>
</table>
Dimethyl sulfoxide (DMSO) was used as the negative control in the antimicrobial screening test. The *in vitro* antimicrobial screening to DMSO showed that there was no susceptibility to any of the microbes (Table 5).

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>ZONE OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20% v/v DMSO</td>
</tr>
<tr>
<td>S. typhi</td>
<td>NS</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>NS</td>
</tr>
<tr>
<td>S. aureus</td>
<td>NS</td>
</tr>
<tr>
<td>P. aeuruginosa</td>
<td>NS</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>NS</td>
</tr>
<tr>
<td>E. coli</td>
<td>NS</td>
</tr>
</tbody>
</table>

### 2.13 SOLUBILITY TESTS

The solubility tests revealed that the crude sap of *P. angolensis* was soluble in 50% ethanol. The aqueous extract of *C. sanguinolenta* was soluble in water and the ethanol form of *C. sanguinolenta* is soluble in 50% ethanol (Table 6).

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Solubility status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. angolensis</em> (sap)</td>
<td>Soluble in a 50:50 ratio of ethanol to water</td>
</tr>
<tr>
<td><em>C. sanguinolenta</em> (aqueous)</td>
<td>Soluble in water</td>
</tr>
<tr>
<td><em>C. sanguinolenta</em> (ethanol)</td>
<td>Soluble in a 50:50 ratio of ethanol to water</td>
</tr>
</tbody>
</table>

### 2.14 TOTAL PHENOL CONTENT

The plot of mean absorbances against the final concentrations indicated the standard curve of pyrogallic acid. Based on Fig. 2, the total phenol concentrations present in the various extracts were extrapolated using their absorbances.

The concentration-mean absorbance calibration for the ten stock concentrations of the standard pyrogallic acid (Fig. 2) revealed that the mean absorbances of the standard were in the range of 0.065 to
1.562 for the concentration range of 0.020 mg/mL to 10 mg/mL. From this study, *P. angolensis* (sap), *C. sanguinolenta* (aqueous), and *C. sanguinolenta* (ethanol) recorded values of 55.427 ± 4.248 SEM, 11.642 ± 4.248 SEM, and 26.888 ± 4.248 SEM, respectively (Table 7). The p-value of 0.0001, which was obtained, indicated a statistical difference.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC (g/100g GAE ± SEM)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. angolensis</em> (sap)</td>
<td>55.427 ± 4.248</td>
<td>p = 0.0001</td>
</tr>
<tr>
<td><em>C. sanguinolenta</em> (aqueous)</td>
<td>11.642 ± 4.248</td>
<td></td>
</tr>
<tr>
<td><em>C. sanguinolenta</em> (ethanol)</td>
<td>26.888 ± 4.248</td>
<td></td>
</tr>
</tbody>
</table>

### 2.15 ANTIOXIDANT ACTIVITY

Figures 3-6 show the plots of the percentage scavenging activity of the various extracts as well as the standard against their respective concentrations and the respective IC$_{50}$ values were obtained. It was observed that the *P. angolensis* (sap) recorded a value of 0.0674 mg/mL which was closest to the value of the BHT standard (0.0432 mg/mL). *C. sanguinolenta* (ethanol) and *C. sanguinolenta* (aqueous) recorded IC$_{50}$ values of 1.002 mg/mL and 2.1609 mg/mL, respectively.

### 2.16 PHYTOCONSTITUENTS SCREENED

The phytochemical screening results obtained from the root of *P. angolensis* and the root of *C. sanguinolenta* are represented in Table 8. Six of the phytoconstituents were identified in *P. angolensis* except for flavonoids that were not identified in the crude extract, while in the case of the aqueous and ethanolic extracts of *C. sanguinolenta*, alkaloids, cardiac glycosides, and saponins were identified.
Table 8
Phytoconstituents of the sap of *P. angolensis* and the root of *C. sanguinolenta*

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sap of <em>P. angolensis</em></th>
<th>Aq. extract of <em>C. sanguinolenta</em></th>
<th>EtOH extract of <em>C. sanguinolenta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytochemicals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

‘-’ Not detected ‘+’ detected

EtOH = Ethanol
Aq = Aqueous

Discussion

Antimicrobial Resistance (AMR) is a global health issue that causes significant mortality and morbidity. Multiple drug resistance in Gram-positive and Gram-negative bacteria has made treating common illnesses with standard medicines challenging (27). The rapid proliferation of multiple drug resistance bacteria, along with a lack of effective medications and suitable preventative measures, has prompted the development of innovative treatment alternatives and alternative antimicrobial treatments that are both less expensive and more effective (27).

Bioactive plant constituents have been used in the treatment of both Gram-positive and Gram-negative bacterial infections (28) for centuries of years. Several studies in the West African sub-region have reported that *C. sanguinolenta* is an anti-malarial plant (11, 12). There have been other reports on its antimicrobial properties by researchers such as Boakye-Yiadom, Mills-Robertson *et al.*, and Paulo *et al.* (11–13, 29).

The current study found that *C. sanguinolenta* was susceptible to *S. aureus* (Gram-positive bacteria) but resistant to Gram-negative organisms such as *P. aeruginosa*, *S. saprophyticus*, *P. mirabilis*, and *S. typhi* (Tables 2 and 3). This finding was consistent with another study by Boakye-Yiadom (29). Boakye-Yiadom (29), revealed that less than 50 mg/mL of aqueous extract generates mild antibacterial activity, a finding that is similar to a later investigation done by Paulo *et al.* (13, 29). This observation could be due to the action of cryptolepine (a bioactive ingredient in *C. sanguinolenta*) on the bacterial cell wall of both Gram-
positive and Gram-negative organisms. Gram-negative bacteria have an outer cell membrane, a lipopolysaccharide with low permeability (30). Some Gram-negative microorganisms also express resistance to inducible cephalosporins or antibiotic efflux pumps that give them high intrinsic resistance to antibiotics; hence, such could be why the extracts of the *C. sanguinolenta* did not work successfully on the selected Gram-negative organisms (30). These current findings contradict the conclusions from Mills-Robertson *et al.* (12), in which the plant extract worked against both Gram-positive and Gram-negative microorganisms used in their studies.

*Pycnanthus angolensis* showed high potency against both selected Gram-negative and Gram-positive organisms except *P. mirabilis*. The potency was concentration and dose-dependent. This current study finding is consistent with a similar study by Chukwudozie and Ezeonu (21). Chukwudozie and Ezeonu (21), reported that the stem bark of *P. angolensis* showed higher inhibition when tested against Gram-positive and negative bacteria. In those studies, the ethanol extracts of the plant extracts were more susceptible than the aqueous extracts when used against the selected microorganisms (21). The resistance of *P. mirabilis* could be due to dose dependency and, therefore, will require a higher dose of the plant extract to be susceptible (27). The current study shows that the phytochemicals in *P. angolensis* are potent against both Gram-negative and Gram-positive organisms in a dose-dependent pattern. It will be ideal to investigate this further in developing novel antimicrobial agents to tackle the growing threat of AMR (27).

Results obtained from *P. angolensis* has confirmed the early claims by Omobuwajo *et al.* (10) and Sofidiya and Awolesi (6) (Table 1), that *P. angolensis* is a remedy for chest pains and skin diseases such as boils, furuncles caused by *S. aureus*, wound healing and gastrointestinal ailment which are usually caused by some of these microorganisms (6, 10). Agayare *et al.* (9), and Onocho and Otula (15) also claimed that *P. angolensis* is a medicinal source for the management of food poisoning, bloody diarrhoea, and urinary tract infections caused by *S. typhi* and *S. saprophyticus*.

Phenols have been reported to have antiseptic, anti-inflammatory, antimicrobial, and anti-tumour properties, and tannins have also been reported to have anti-ageing properties as well as skin regeneration, anti-inflammatory and diuretic properties (31). According to Agyare *et al.* (15), flavonoids have splendid antimicrobial and anticancer activities, while alkaloids are used as painkiller medications (32).

Extraction is necessary to separate plants’ insoluble residues from their soluble active components. (33). It is one of the main steps in obtaining phytochemicals for use as supplements, food ingredients, drugs, and cosmetics (34). The aqueous and ethanolic extracts of *C. sanguinolenta* were prepared using the decoction process with water and ethanol as the solvents, respectively. They were used because they served as the most suitable solvent for the extraction process due to their differences in the polarity of the phytochemicals present and their safety for human consumption (35). Water is known to be a universal polar solvent, and it is therefore capable of dissolving numerous solubilizing substances. It also showed very effective performance in some studies on extraction and had the upper hand over all other solvents.
due to its unlimited usage. On the other hand, ethanol is a less polar solvent (due to its covalent bonds) and one of the most widely used in antioxidant extraction due to its generally recognized safe solvent ability, a term known as the "GRAS ability of ethanol" (36–38). The addition of water to ethanol also dramatically increases the rate of extraction of metabolites (39). Moreover, both solvents are massively used by herbalists in the preparation of herbal medicine despite their inadequate or no knowledge of the effects of their use on the active components in the plants involved; hence, the use of water and ethanol for this study (40).

The Folin-Ciocalteu reagent assay is the most readily available and straightforward method for determining the total phenol contents present in samples. The yellow reagent comprises phosphomolybdic and phosphotungstic acids, which are heterocyclic acids with molybdenum and tungsten in an oxidation state of +6 (41). The assay works on the principle that, under primary conditions (that is, the addition of sodium carbonate), phenolic compounds if present, dissociate to form a phenolate ion, which reduces the phosphomolybdic and phosphotungstic acids of the Folin-Ciocalteu reagent to form molybdenum blue and tungsten blue with a mean oxidation state between 5 and 6 (41). These products are chromogens; thus, the blue colour they produce and their intensity can be measured using absorbance readings from a spectrophotometer with wavelength ranges between 500 nm and 760 nm (41). This principle is a typical oxidation-reduction reaction, and this study considered absorbance readings at 750 nm (41). Results from this study (Table 7) revealed that the crude sap of *P. angolensis* contained the highest amount of total phenol content compared to the roots of *C. sanguinolenta*. In comparison, it was observed that the ethanol extract had a significantly higher total phenol content than the aqueous solution. The aqueous solution recorded a low value, possibly due to the inability of water to adequately extract non-polar polyphenols into the solution (42). In the current study, 70% ethanol was also used to prepare the ethanol extract. Combined with water, ethanol has a much greater potential to extract polar and non-polar polyphenols into solution than it would if it were used alone (that is, at 100% ethanol) (42). Low values were also recorded in the aqueous extract, possibly due to the action of the enzyme polyphenol oxidase, which works best in an aqueous medium and acts on polyphenols and degrades them, thereby reducing their presence in solution (42). From the hypothesis test carried out, it was realised that a comparison of the three extracts produced a p-value of 0.0001, indicating that the various extracts were very different from each other and, as such, one extract could not be substituted for another for its usage in the manufacturing of potent drugs (40).

This study showed an increase in the mean percentage of antioxidant activity as the concentrations increased. This was reflected in all the extracts and the standard BHT to which the extracts were compared. It was observed that *P. angolensis* (sap), *C. sanguinolenta* (aqueous), and *C. sanguinolenta* (ethanol) recorded IC$_{50}$ values of 0.0674 mg/mL, 2.1609 mg/mL and 1.002 mg/mL, respectively, compared to the BHT of 0.0432 mg/mL (Figs. 3–6). Comparing the extracts for the study to the standard, *P. angolensis* (sap) recorded values comparable to the reference value (43). Even though the IC$_{50}$ values of the aqueous and ethanol forms of *C. sanguinolenta* are not close to that of the standard, it can be said conclusively that they are good antioxidants as few amounts of these extracts can mop up 50% of free
radicals (44). It was also observed that *C. sanguinolenta* (ethanolic) recorded an IC$_{50}$ value much closer to the standard than *C. sanguinolenta* (aqueous). This indicates that the ethanol crude extract of *C. sanguinolenta* is a much better antioxidant than the aqueous extract. This is likely due to the percentage of ethanol (70%) used for the extraction. Coupled with some amount of water, ethanol had a more significant potential to dissolve more phenolic compounds than using only distilled water or ethanol (45). It must be noted, that the closer an IC$_{50}$ value of an extract is to zero, the more likely it is for the extract to possess potent antioxidant capabilities (24). Thus, the crude sap of *P. angolensis* was a more powerful antioxidant than the ethanol extract of *C. sanguinolenta*, which was also a better antioxidant than the aqueous extract of *C. sanguinolenta*. On the whole, all three extracts proved to be very effective antioxidants. The current study findings agree with study by Khanc *et al.* (46), where it was reported that in the nitric oxide scavenging experiment, the crude extract of *P angolensis* showed astounding efficacy with a 99.0% Radical Scavenging Activity (RSA) compared to the reference, n-propyl gallate (90.3% RSA) (46). Another study conducted by Oladimeji and Akpan (7) also showed that *P angolensis* had a moderate antioxidant activity of 0.55 µg/mL when compared with the standard drug (Vitamin C) with an antioxidant activity of 0.45 µg/mL (7). Furthermore, the antioxidant activities of *P angolensis* were better than those of vitamins A and E at 0.57 and 0.59 µg/mL (7), respectively. The antioxidant capabilities of the extracts were instructive since the phytochemical analysis of the plants revealed the presence of terpenes, flavonoids, and tannins, all of which have antioxidant properties. Studies have shown a direct correlation between the total phenol content and extracts' antioxidant activity (47–49). It is therefore not surprising that the crude sap of *P angolensis*, which recorded a higher IC$_{50}$ value of 0.0674 mg/mL, had a higher amount of phenol content (55.427 ± 4.248) compared to the ethanol extract of *C. sanguinolenta* which also recorded a higher IC$_{50}$ value of 1.002 mg/mL and an amount of 26.888 ± 4.248 g/100g GAE of phenol content than its aqueous extract which recorded the least values (11, 12).

The present work revealed that the root extracts (aqueous and ethanolic) of *C. sanguinolenta* possess alkaloids, cardiac glycosides, and saponins. In contrast, the crude sap of *P. angolensis* possessed alkaloids, cardiac glycosides, tannins, saponins, terpenoids, and phenols. These secondary metabolites have been found to possess antimicrobial and anticancer properties. Alkaloids are mostly known for their toxicity against cells of foreign organisms, and these have the potential to eliminate and reduce human cancer cell lines (15). Alkaloids are naturally occurring metabolites in plants and are mostly present as heterocyclic compounds containing nitrogen atoms (which are very essential for plant growth) and are in the form of salts coupled with organic acids (15). It was therefore not surprising that they were found to be present in various extracts. Eleazu and Eleazu(50) reported that isolated alkaloids and their derivatives possess medicinal properties due to their antispasmodic, antibacterial, and analgesic properties (50).

Tannins are known to form irreversible complexes with proline-rich proteins (51). Parekh and Chanda (52) also found that tannins react with proteins to produce essential effects for the treatment of inflamed or ulcerated tissues. Plants rich in tannins are astringent and may be used for treating intestinal disorders like dysentery and diarrhoea (52). *P. angolensis* is a plant used to treat intestinal disorders like dysentery and diarrhoea diseases in West Africa. This may prove the antimicrobial and anticancer properties of *P*
*angolensis* based on its phytochemical constituents (11). The absence of flavonoids in both aqueous and ethanolic extracts of the root extracts of *C. sanguinolenta* and crude sap of *P. angolensis* does not mean a lack of bioactive constituents (12). However, this may be due to the low levels of the bioactive compounds in the crude plant extracts used in this current study (12). Saponins possess hypolipidemic and anticancer activities and are also important for co-functioning with cardiac glycosides to enable them to carry out their activities which include serving as cardiac drugs and promoting nitrogen retention in osteoporosis or with animals with wasting illness (53–55). Terpenoids also have a broad range of properties including antitumor, antiviral, bactericidal, fungicidal, analgesic, anti-inflammatory, spermicidal, and cytotoxic activities (56). Phenolic compounds are most notable for their antioxidant action due to their high tendency to chelate metals and inactivate their actions (57). All these medicinal effects of the various phytoconstituents make them possible for their usage in treating numerous diseases.

Results from this current study were consistent with the works carried out by others. Considering the sap of *P. angolensis*, the work done by Udeozo *et al.* (58), on the powdered stem revealed the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, and glycosides. Oladimeji *et al.* (7), who also worked on the ethanolic extract revealed the presence of saponins, cardiac glycosides, and terpenoids except for alkaloids, tannins, and flavonoids. Akinyenye and Olatunya (59) also confirmed positive tests for alkaloids, saponins, tannins, terpenoids, flavonoids, and cardiac glycosides upon working on the aqueous extract of the plant. Their results were also compared with that of Udeozo *et al.* (58). This study was consistent with the works by Mills-Robertson *et al.* (12), who worked on the cold and hot water extracts as well as the ethanol extracts and revealed the presence of alkaloids. Bunalema (60), worked on the crude extracts of the roots and revealed the presence of alkaloids, tannins, and flavones. Claude *et al.* (61) worked on the methanol extracts and obtained positives for alkaloids, tannins, and flavones just as obtained by Bunalema (60). Mills-Robertson *et al.* (11), worked on the aqueous, ethanol, and chloroform extracts that revealed the presence of alkaloids and the absence of saponins and flavonoids in all three extracts. Chahar *et al.* (14), also worked on the aqueous, ethanol, and chloroform extracts and their study revealed the presence of alkaloids and terpenes (for only the aqueous extract) and the absence of saponins and flavonoids in all the three extracts. A study carried out by Chime (62) on the ethanol crude extracts of *C. sanguinolenta* revealed the presence of alkaloids, terpenoids and glycosides. Saponins, tannins, but flavonoids were absent. From the various studies on *C. sanguinolenta*, it was realized that alkaloids tested positive throughout and this confirms the work done by Gibbons *et al.* (63), who not only identified the alkaloid cryptolepine but also went a step further to isolate this potent alkaloid. The differences in results from this study as compared to the others could be genuinely due to their absence or the difference in the methods of preparation and the types or parts of crudes used in the various extracts (11).

**Conclusion**

This study provides valuable insight into the potential antimicrobial properties and total phenolic content of two selected West African plants, *P. angolensis* and *C. sanguinolenta*. The *in vitro* results suggest that these plant extracts may have promising applications in the treatment of microbial infections.
Recommendation

Further research is necessary to understand the mechanisms of action and isolate the bioactive components responsible for the observed effects. Despite this, the findings of this study highlight the potential of *P. angolensis* and *C. sanguinolenta* as excellent candidates for the development of novel antimicrobial agents. With the urgent need for new and effective treatments against microbial infections, these plants could hold the key to a brighter future in the fight against these pathogens.

Declarations

Ethics Approval and Consent to Participate

Ethical approval was not applicable in this study however, instructional, national and international guidelines and legislation was followed in this study.

Consent for Publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding authors upon reasonable request.

Declarations of Competing Interest

The authors declare they have no known competing financial interests and personal relationships that could have appeared to influence the work reported in this paper.

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Authors’ contributions


F.A.A., H.S. and C.Y.A.; investigation, F.A.A. and H.S.; writing-original draft preparation, F.A.A.; writing-review and editing, F.C.M.R. H.S. and C.Y.A.; supervision, F.C.M.R. All authors have read and agreed to be the published version of the manuscript.

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Figures

Figure 1

Concentrations of extracts of *C. sanguinolenta* against *S. aureus*
Figure 2

Standard curve of mean absorbances against the concentration of pyro-gallic acid
Figure 3

Antioxidant activity of BHT

IC$_{50}$ = 0.0432 mg/mL
Figure 4

Antioxidant activity of *P. angolensis* (sap)
Figure 5

Antioxidant activity of *C. sanguinolenta* (aqueous).

\[ \text{IC}_{50} = 2.1609 \text{ mg/mL} \]
Figure 6

Antioxidant activity of *C. sanguinolenta* (ethanol).